TECHNICAL MANUSCRIPT 270

ORAL LIVE TULAREMIA VACCINE

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DECEMBER 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

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Fort Detrick, Frederick, Maryland

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Project 1C522301A059

December 1965

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In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

It has been established that live tularemia vaccine prepared at Fort Detrick and administered dermally by acupuncture or aerogenically to animals and man is innocuous and immunologically superior to killed preparations. Data on the pathogenesis and immunogenicity of live vaccine strain LVS have been sufficiently encouraging to warrant an evaluation of oral vaccination with this strain. A preliminary test of the oral infectivity of highly virulent strain SCHU for the monkey (Macaca mulatta) revealed that 10^8 organisms mixed in milk and swallowed infected two of eight animals, both of which died; a dose of 10^4 cells did not result in any evidence of infection. Doses of 10^8 and 10^{10} organisms infected all monkeys tested and the majority died; comparable results were obtained when 10^{10} organisms in gelatin capsules were swallowed intact. In another study, groups of 16 monkeys swallowed 10^8 to 10^{10} live organisms of strain LVS or strain 425 in milk; strain 425 is incapacitating but seldom lethal for the monkey when inoculated dermally or aerogenically. A group of eight monkeys were administered strain LVS dermally by acupuncture. Both the oral and the dermal inocula of LVS proved innocuous but 13 of the 16 animals that received strain 425 showed a transient low-grade febrile reaction. An agglutinin response was obtained in all vaccinees. Approximately 10 weeks after vaccination, these animals and a group of seven unvaccinated controls inhaled 10^4 organisms of strain SCHU. All animals became infected but 64 to 71% of the vaccinees survived in contrast to 14% of the controls. These studies indicate a potential for oral immunization with live tularemia vaccine prepared from strain LVS.

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Groups of five to eight conditioned animals were fed 10^4, 10^6, 10^8, or 10^{10} live SCHU organisms mixed in milk. A group of five animals received 10^{10} organisms in gelatin capsules to ensure that contamination of the upper respiratory tract might not alone produce infection.

Rectal temperatures were recorded twice daily several days prior to exposure and for 30 days after exposure; weights were recorded weekly. C-reactive protein and sedimentation rates were determined on heparinized blood samples drawn at various intervals before and after ingestion of the test organisms. Serological studies were conducted on serum samples weekly. Animals were frequently observed for clinical signs of illness; i.e., anorexia, diarrhea, weakness, etc.

As indicated in Table 1, the oral ID_{50} of strain SCHU was approximately 10^7 cells. Criteria for infection included febrile response, clinical disease, and serology. Monkeys administered 10^4 cells did not become infected. Twenty-five per cent of the animals fed 10^6 organisms became infected and died. At the 10^8 dose, all animals became infected and 37% survived. All animals receiving 10^{10} SCHU, whether the organisms were fed with milk or contained in a capsule, became infected and died. As indicated, when the dose was increased, time to death was decreased appreciably. Only subtle differences were observed in the course of clinical tularemia initiated by organisms fed in milk or swallowed in a capsule. Animals fed organisms in milk became febrile and anorectic approximately 12 to 24 hours before and died on the average 2 days prior to those that swallowed the capsule.

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* This new designation has been made to honor Dr. Edward Francis of the U.S. Public Health Service and will be used in the next edition of Bergey's Manual.

TABLE 1. ORAL INFECTIVITY OF FRANCISELLA TULARENSIS STRAIN SCHU FOR THE MONKEY

Oral Dose[a]	Infected, %	Dead, %	Average Time to Death, days
10^4	0	0	
10^6	25	25	18
10^8	100	63	11
10^{10}	100	100	5
10^{10} [b]	100	100	7

a. Five to eight animals per group.
b. Organisms contained in gelatin capsules.

Considerable differences in pathologic changes were observed in animals dying from the various oral doses of strain SCHU. Ingestion of 10^6 to 10^8 cells produced lesions in the lung and other organs comparable to those usually observed after an aerogenic exposure; only one animal exhibited inflammation of the large intestine. In contrast, all animals administered 10^{10} organisms showed severe inflammation and ulceration of the intestinal mucosa, especially in the ileum. The majority of these monkeys also showed severe inflammation and circumscribed ulceration of the cardiac region of the stomach. Prior to death, these animals passed clotted blood rectally. F. tularensis was routinely isolated from the mesenteric lymph nodes.

These data on the oral infectivity and pathogenicity of strain SCHU suggested that it might also be possible to obtain an antigenic response in the monkey by administering orally F. tularensis strains of lesser virulence than strain SCHU. Two strains were selected, live vaccine strain LVS, which is immunogenic but innocuous for the monkey when administered by the dermal or respiratory route, and strain 425, which is incapacitating for the monkey but seldom lethal. Groups of 16 monkeys were fed 10^6 to 10^{10} live organisms of strain LVS or 425 in milk. Another group of 8 monkeys was administered strain LVS dermally by acupuncture. Eight animals were used as nonvaccinated controls.

One animal fed LVS showed a transient low-grade fever. With this exception, none of the animals vaccinated with LVS exhibited overt signs of disease. The majority of the animals fed strain 425 showed a transient low-grade febrile response but no other signs of disease. C-reactive protein and sedimentation rate data were not indicative of an active disease process.

Although a positive serological response was detected earlier in animals vaccinated dermally with LVS than in those vaccinated orally with LVS or 425, all vaccinees developed F. tularensis agglutinins (Figure 1). In general, titers were higher in animals that received strain 425. Dermal and oral administration of LVS produced comparable agglutinin titers. Gel diffusion studies showed comparable precipitin production by animals vaccinated dermally or orally with LVS, but animals administered strain 425 orally showed more and denser precipitin bands.

In contrast to the severe disease associated with oral administration of strain SCHU, strain LVS or 425 resulted in only a benign response. Two of the animals fed strain LVS, two fed strain 425, and one vaccinated percutaneously were euthanized approximately 7 weeks after exposure; no pathologic residue were observed. F. tularensis was not isolated from the tissues examined and all organs appeared normal.

Approximately 10 weeks after vaccination animals were challenged via the respiratory route with 10^4 organisms of strain SCHU. On the basis of our previous studies on live tularemia vaccine, it was estimated that this formidable aerogenic dose would result in the infection of all dermal vaccinees with a survival of 50 to 60%. Results presented in Table 2 indicate that although all vaccinees and controls became ill following challenge, 64 to 71% of the vaccinees survived in comparison to 14% of the controls. Vaccinated survivors were febrile for 3 to 14 days and anorectic for 1 to 3 days during the acute phase of disease. The sole surviving control animal experienced a severe illness and appeared moribund for several days. No appreciable differences in the grade of immunity was observed between animals vaccinated with strain LVS and with strain 425, nor between animals vaccinated orally and dermally. These results indicate a potential for effective oral immunization against tularemia.

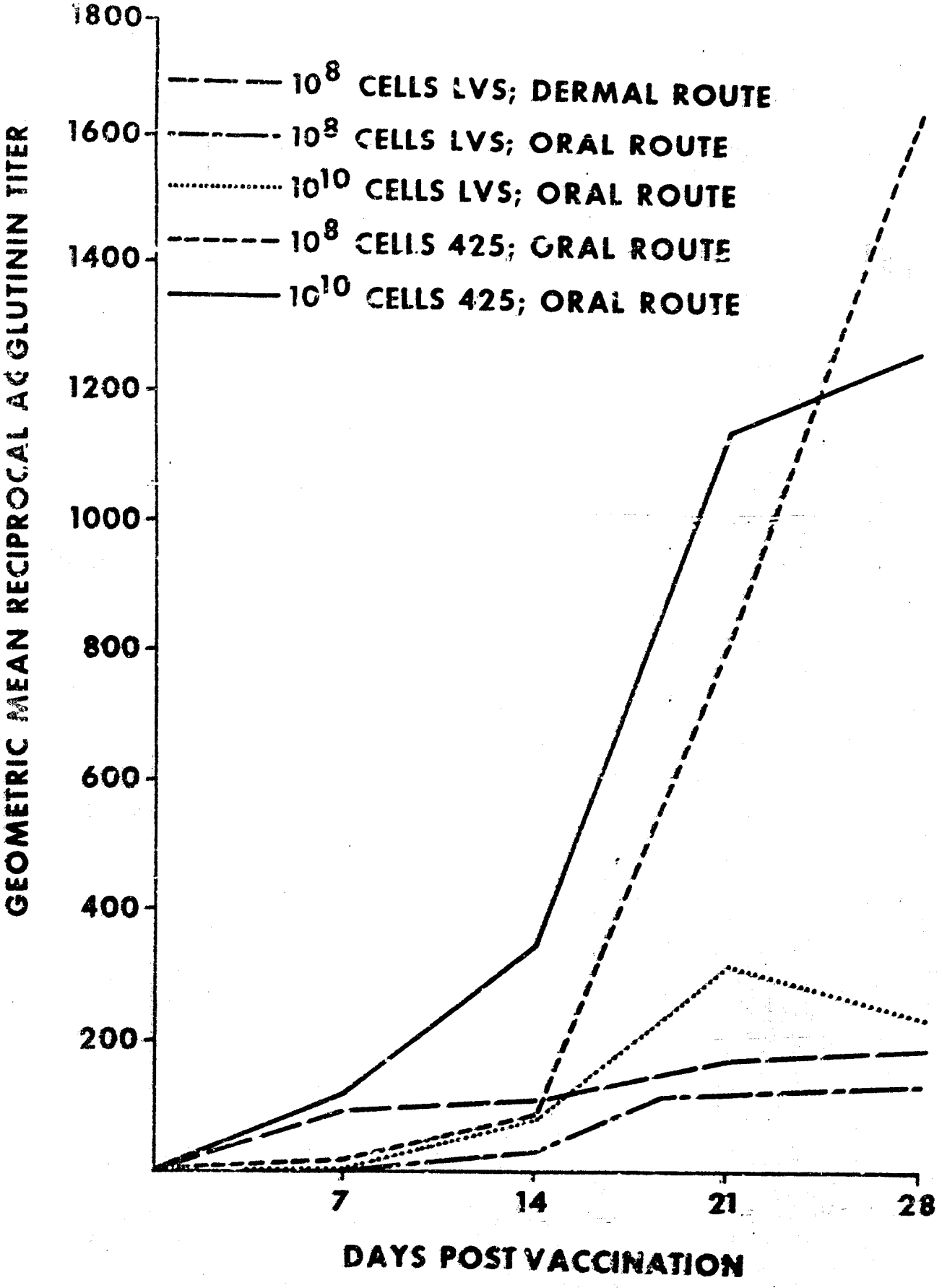

Figure 1. Agglutinin Response of Monkeys Administered *Francisella tularensis* Strains LVS or 425.

TABLE 2. ORAL IMMUNOGENICITY OF FRANCISELLA TULARENSIS LVS AND 425 FOR THE MONKEY

Vaccine Strain	Route	Vaccinees Challenged[a]	Animals Showing Disease				Survival, %
			Mild	Moderate	Severe	Fatal	
LVS	Dermal	7	3	2	0	2	71
LVS	Oral	14	6	3	0	5	64
425	Oral	14	9	1	0	4	71
None		7	0	0	1	6	14

a. 10^4 cells of strain SCHU via the respiratory route.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

U.S. Army Biological Laboratories
Fort Detrick, Frederick, Maryland 21701

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

ORAL LIVE TULAREMIA VACCINE

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(Last name, first name, initial)*

Eigelsbach, Henry T. Hornick, Richard B.
Tulis, Jerry J.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1965	12	0

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1C522301A059	Technical Manuscript 270
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. AVAILABILITY/LIMITATION NOTICES

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Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland 21701

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DD FORM 1 JAN 64 1473

Unclassified
Security Classification